Attorney's Docket No.: 09010-010003 / DIVER 1180-2 Applicant: Dan E. Robertson et al.

Serial No.: 09/903,410 : July 10, 2001 Filed

Page : 2 of 26

## Amendment to the Claims:

Please cancel claims 69 to 72, without prejudice.

Please amend the claims as follows:

This listing of claims will replace all prior versions, and listing, of claims in the application:

## Listing of Claims:

Claim 1 (currently amended): An isolated or recombinant nucleic acid comprising (a) a sequence having at least about 70% [[50%]] sequence identity to SEQ ID NO:26 [[and SEQ ID NO:29]], and encoding a polypeptide having an esterase activity, or, (b) a sequence complementary to (a).

Claim 2 (currently amended): An isolated or recombinant nucleic acid of claim 1, comprising a sequence [[selected from the group consisting of]] comprising SEQ ID NO:26, [[SEO ID NO:29,]] and sequences complementary thereto.

Claim 3 (currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid [[of claim 1]] comprising (a) a sequence having at least about 70% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, and, (b) sequences complementary to (a), under conditions [[of high stringency]] comprising about 50% formamide at about 37°C to 42°C, 5X SSPE, 0.3% SDS, and 200 n/ml sheared and denatured salmon sperm DNA.

Claim 4 (currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid [[of claim 1]] comprising (a) a sequence having at least about 70% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, and, (b) sequences complementary to (a), under conditions [[of moderate stringency]] comprising a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na<sub>2</sub>EDTA containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T<sub>m</sub>-10°C.

Serial No.: 09/903,410 Filed: July 10, 2001

Page : 3 of 26

Claim 5 (currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid [[of claim 1]] comprising (a) a sequence having at least about 70% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, and, (b) sequences complementary to (a), under conditions [[of low stringency]] comprising about 35% formamide at about 35°C to 42°C, 5X SSPE, 0.3% SDS, and 200 n/ml sheared and denatured salmon sperm DNA.

Claim 6 (currently amended): An isolated or recombinant nucleic acid having at least 55% sequence identity to the nucleic acid of claim 103 1 as determined by analysis with a sequence comparison algorithm.

Claim 7 (currently amended): An isolated or recombinant nucleic acid having at least 60% sequence identity to the nucleic acid of claim 103 1 as determined by analysis with a sequence comparison algorithm.

Claim 8 (currently amended): An isolated or recombinant nucleic acid having at least 65% sequence identity to the nucleic acid of claim 7 1 as determined by analysis with a sequence comparison algorithm.

Claim 9 (currently amended): An isolated or recombinant nucleic acid having at least 70% sequence identity to the nucleic acid of claim 8 [[1]] as determined by analysis with a sequence comparison algorithm.

Claim 10 (currently amended): An isolated or recombinant nucleic acid having at least 75% sequence identity to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.

Claim 11 (currently amended): An isolated or recombinant nucleic acid having at least 80% sequence identity to the nucleic acid of claim 10 1 as determined by analysis with a

Serial No.: 09/903,410 : July 10, 2001

: 4 of 26 Page

sequence comparison algorithm.

Claim 12 (currently amended): An isolated or recombinant nucleic acid having at least 85% sequence identity to the nucleic acid of claim 11 1 determined by analysis with a sequence comparison algorithm.

Claim 13 (currently amended): An isolated or recombinant nucleic acid having at least 90% sequence identity to the nucleic acid of claim 12 1 determined by analysis with a sequence comparison algorithm.

Claim 14 (currently amended): An isolated or recombinant nucleic acid having at least 95% sequence identity to the nucleic acid of claim 13 1 determined by analysis with a sequence comparison algorithm.

Claim 15 (previously presented): The isolated or recombinant nucleic acid of claim 1, wherein the sequence comparison algorithm is FASTA version 3.0t78 with the default parameters.

Claim 16 (currently amended): An isolated or recombinant nucleic acid comprising (a) at least 30 [[10]] consecutive bases of a sequence as set forth in SEQ ID NO:26 or [[SEO ID NO:29]], at least 30 [[10]] consecutive bases of a sequence having at least 70% identity to SEQ ID NO:26 [[or SEQ ID NO:29]] and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a) [[thereto]].

Claim 17 (currently amended): The [[An]] isolated or recombinant nucleic acid of claim 16, wherein the [[having at least about 50%]] sequence identity [[to the nucleic acid of claim 16 as]] is determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

Claim 18 (currently amended): The [[An]] isolated or recombinant nucleic acid

Serial No.: 09/903,410 Filed: July 10, 2001

Page : 5 of 26

of claim 16, wherein the [[having at least about 50%]] sequence identity [[to the nucleic acid of claim 16 as]] is determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

Claim 19 (currently amended): The [[An]] isolated or recombinant nucleic acid of claim 16, wherein the sequence identity is [[having]] at least about 90% 60% sequence identity to the nucleic acid of claim 16 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

Claim 20 (currently amended): The [[An]] isolated or recombinant nucleic acid of claim 19, wherein the sequence identity is [[having]] at least about 95% 65% sequence identity to the nucleic acid of claim 16 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

Claim 21 (currently amended): <u>The [[An]]</u> isolated or recombinant nucleic acid of claim 20, wherein the sequence identity is [[having]] at least about <u>97%</u> 65% sequence identity to the nucleic acid of claim 16 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

Claim 22 (currently amended): An isolated or recombinant nucleic acid encoding (a) a polypeptide having an esterase activity and having at least 70% sequence identity to a sequence as set forth in [[selected from the group consisting of]] SEQ ID NO:36 [[and SEQ ID NO:39]], or, (b) enzymatically active fragments of (a).

Claim 23 (currently amended): An isolated or recombinant nucleic acid encoding a polypeptide comprising at least 30 [[10]] consecutive amino acids of a polypeptide having an esterase activity and having at least 70% sequence identity to a sequence as set forth in [[selected from the group consisting of]] SEQ ID NO:36 [[and SEQ ID NO:39]].

Claims 24 to 39 (canceled)

Serial No.: 09/903,410 Filed: July 10, 2001 Page: 6 of 26

Claim 40 (previously presented): A method of producing a polypeptide having an esterase activity comprising introducing a nucleic acid as set forth in claim 1 into a host cell under conditions that allow expression of the nucleic acid to produce a polypeptide.

Claim 41 (currently amended): A method of producing a polypeptide comprising at least 30 [[10]] amino acids of a sequence as set forth in SEQ ID NO:36 [[or SEQ ID NO:39]] or at least 30 [[10]] amino acids of a sequence encoded by a nucleic acid as set forth in claim 1, comprising introducing a nucleic acid encoding the polypeptide, operably linked to a promoter, into a host cell under conditions that allow expression of the polypeptide.

Claim 42 (currently amended): A method of generating a variant comprising: obtaining a nucleic acid comprising a sequence as set forth in SEQ ID NO:26 [[or SEQ ID NO:29]], or a sequence as set forth in claim 1, or, sequences complementary thereto, or fragments comprising at least 30 consecutive nucleotides thereof, or fragments comprising at least 30 consecutive nucleotides of the sequences complementary to SEQ ID NO:26 [[or SEQ ID NO:29]]; and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence.

Claim 43 (original): The method of claim 42, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis and any combination thereof.

Claim 44 (original): The method of claim 42, wherein the modifications are introduced by error-prone PCR.

Serial No. : 09/903,410 Filed : July 10, 2001 Page : 7 of 26

1 480

Claim 45 (original): The method of claim 42, wherein the modifications are introduced by shuffling.

Claim 46 (original): The method of claim 42, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

Claim 47 (original): The method of claim 42, wherein the modifications are introduced by assembly PCR.

Claim 48 (original): The method of claim 42, wherein the modifications are introduced by sexual PCR mutagenesis.

Claim 49 (original): The method of claim 42, wherein the modifications are introduced by *in vivo* mutagenesis.

Claim 50 (original): The method of claim 42, wherein the modifications are introduced by cassette mutagenesis.

Claim 51 (original): The method of claim 42, wherein the modifications are introduced by recursive ensemble mutagenesis.

Claim 52 (original): The method of claim 42, wherein the modifications are introduced by exponential ensemble mutagenesis.

Claim 53 (original): The method of claim 42, wherein the modifications are introduced by site-specific mutagenesis.

Claim 54 (original): The method of claim 42, wherein the modifications are introduced by gene reassembly.

Attorney's Docket No.: 09010-010003 / DIVER 1180-2 Applicant: Dan E. Robertson et al.

Serial No.: 09/903,410 : July 10, 2001 Filed

Page : 8 of 26

Claim 55 (original): The method of claim 42, wherein the modifications are introduced by gene site saturated mutagenesis.

Claims 56 to 60 (canceled)

Claim 61 (currently amended): A method for comparing a first sequence to a reference sequence wherein said first sequence comprises (a) [[is]] a nucleic acid sequence as set forth in SEQ ID NO:26 [[or SEQ ID NO:29]] or (b) a sequence [[sequences]] having at least 70% [[50%]] sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26 [[thereto]], or (c) a sequence complementary to (a) or (b), or, (d) a polypeptide sequence as set forth in SEQ ID NO:36 [[or SEQ ID NO:39]] or (e) a sequence [[sequences]] having at least 70% [[50%]] sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26, the method [[thereto]] comprising the following steps:

reading the first sequence and the reference sequence through use of a computer program which compares sequences; and

determining differences between the first sequence and the reference sequence with the computer program.

Claim 62 (original): The method of claim 61, wherein determining differences between the first sequence and the reference sequence comprises identifying polymorphisms.

Claim 63 (currently amended): A method for identifying a feature in (a) a sequence as set forth in SEQ ID NO:26 [[or SEQ ID NO:29]] or, (b) sequences having at least 70% [[50%]] sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26 [[thereto]], or (c) a sequence complementary to (a) or (b), or, (d) a polypeptide sequence as set forth in SEQ ID NO:36 [[or SEO ID NO:39]] or (e) having at least 70% [[50%]] sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26, the method [[thereto]] comprising the following steps:

reading the sequence through the use of a computer program which identifies

Serial No.: 09/903,410 Filed: July 10, 2001

Page : 9 of 26

features in sequences; and

identifying features in the sequences with the computer program.

Claim 64 (canceled)

Claim 65 (previously presented): A method of catalyzing the hydrolysis of an ester comprising contacting a sample containing an esterase with a polypeptide encoded by a sequence as set forth in claim 1 under conditions which facilitate the hydrolysis of the ester.

Claim 66 (canceled)

Claim 67 (Currently amended): A nucleic acid probe comprising an oligonucleotide from about 10 to 50 nucleotides in length or at least about [[10, 15, 20, 25,]] 30, 35, 40, 45, 50, 75, 100, 150 or 200 nucleotides in length and having an area of at least [[10]] 30 contiguous nucleotides of (a) a sequence having at least [[50%]] 70% sequence identity to a nucleic acid as set forth in SEQ ID NO:26 [[claim 1]] or (b) a [[its complementary]] sequence complementary to (a), and which hybridizes to the nucleic acid under moderate or highly stringent conditions to form a detectable target:probe duplex.

Claim 68 (currently amended): The probe of claim 67, wherein the oligonucleotide [[is]] comprises DNA or RNA.

Claims 69 to 72 (canceled)

Claim 73 (previously presented): The probe of claim 67, wherein the sequence in (a) has [[having]] at least 75% sequence identity to the nucleic acid.

Claim 74 (currently amended): The probe of claim <u>73</u> [[67]], <u>wherein the sequence in (a) has [[having]]</u> at least 80% sequence identity to the nucleic acid.

Serial No.: 09/903,410 Filed: July 10, 2001 Page: 10 of 26

Claim 75 (currently amended): The probe of claim <u>74</u> [[67]], <u>wherein the</u> sequence in (a) has [[having]] at least 80% sequence identity to the nucleic acid.

Claim 76 (currently amended): The probe of claim <u>75</u> [[67]], <u>wherein the</u> sequence in (a) has [[having]] at least 90% sequence identity to the nucleic acid.

Claim 77 (currently amended): The probe of claim <u>76</u> [[67]], <u>wherein the</u> sequence in (a) has [[having]] at least 95% sequence identity to the nucleic acid.

Claim 78 (currently amended): The probe of claim <u>77</u> [[67]], which is fully complementary to the nucleic acid.

Claim 79 (original): The probe of claim 67, wherein the oligonucleotide is 15-50 bases in length.

Claim 80 (original): The probe of claim 67, wherein the probe further comprises a detectable isotopic label.

Claim 81 (original): The probe of claim 67, wherein the probe further comprises a detectable non-isotopic label selected from the group consisting of a fluorescent molecule, a chemiluminescent molecule, an enzyme, a cofactor, an enzyme substrate, and a hapten.

Claim 82 (currently amended): A nucleic acid probe comprising an oligonucleotide from about 10 to 50 nucleotides in length or at least about [[10, 15,]] 20 [[, 25, 30, 35, 40, 45, 50, 75, 100, 150 or 200]] nucleotides in length and having an area of at least [[15]] 20 contiguous nucleotides of a sequence (a) having at least 90% sequence identity to a nucleic acid as set forth in SEQ ID NO:26, [[claim 1]] or, (b) its complementary sequence, and which hybridizes to the nucleic acid under moderate or to highly stringent conditions to form a detectable target:probe duplex.

Serial No.: 09/903,410 Filed: July 10, 2001

Page : 11 of 26

Claim 83 (currently amended): A nucleic acid probe comprising an oligonucleotide from about 10 to 50 nucleotides in length or at least about [[10, 15, 20, 25,]] 30, 35, 40, 45, 50, 75, 100, 150 or 200 nucleotides in length and having an area of at least [[15]] 20 contiguous nucleotides of a sequence (a) having at least 95% sequence identity to a nucleic acid as set forth in SEQ ID NO:26, [[claim 1]] or, (b) its complementary sequence, and which hybridizes to the nucleic acid under moderate or to highly stringent conditions to form a detectable target:probe duplex.

Claim 84 (currently amended): A nucleic acid probe comprising an oligonucleotide from about 10 to 50 nucleotides in length or at least about [[10, 15, 20, 25,]] 30, 35, 40, 45, 50, 75, 100, 150 or 200 nucleotides in length and having an area of at least 15 contiguous nucleotides of a sequence (a) having at least 97% sequence identity to a nucleic acid as set forth in SEQ ID NO:26, [[claim 1]] or, (b) its complementary sequence, and which hybridizes to the nucleic acid under moderate or to highly stringent conditions to form a detectable target:probe duplex.

Claim 85 (currently amended): A polynucleotide probe for isolation or identification of esterase genes having a sequence which is the same as or fully complementary to at least a portion of SEQ ID NO:26 [[or SEQ ID NO:29]].

Claim 86 and 87 (canceled)

Claim 88 (currently amended): A method for modifying small molecules, comprising mixing a polypeptide encoded by a polynucleotide of claim 1 or enzymatically active fragments thereof with a small molecule to produce a modified small molecule.

Claim 89 (original): The method of claim 88 wherein a library of modified small molecules is tested to determine if a modified small molecule is present within the library which exhibits a desired activity.

Serial No.: 09/903,410 : July 10, 2001 Filed

: 12 of 26 Page

Claim 90 (original): The method of claim 89 wherein a specific biocatalytic reaction which produces the modified small molecule of desired activity is identified by systematically eliminating each of the biocatalytic reactions used to produce a portion of the library, and then testing the small molecules produced in the portion of the library for the presence or absence of the modified small molecule with the desired activity.

Claim 91 (original): The method of claim 90 wherein the specific biocatalytic reactions which produce the modified small molecule of desired activity is optionally repeated.

Claim 92 (original): The method of claim 90 or 91 wherein (a) the biocatalytic reactions are conducted with a group of biocatalysts that react with distinct structural moieties found within the structure of a small molecule, (b) each biocatalyst is specific for one structural moiety or a group of related structural moieties; and (c) each biocatalyst reacts with many different small molecules which contain the distinct structural moiety.

Claim 93 (currently amended): An isolated or recombinant nucleic acid having at least about 75% sequence identity to the nucleic acid of claim 16 as determined by BLASTN analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

Claim 94 (currently amended): An isolated or recombinant nucleic acid having at least about 80% sequence identity to the nucleic acid of claim 93 [[16]] as determined by BLASTN analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

Claim 95 (currently amended): An isolated or recombinant nucleic acid having at least about 85% sequence identity to the nucleic acid of claim 94 [[16]] as determined by BLASTN analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

Serial No.: 09/903,410 Filed: July 10, 2001

Page : 13 of 26

Claim 96 (currently amended): An isolated or recombinant nucleic acid having at least about 90% sequence identity to the nucleic acid of claim <u>95</u> [[16]] as determined by <u>BLASTN</u> analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the <u>default parameters</u>.

Claim 97 (currently amended): An [[A]] isolated or recombinant nucleic acid comprising at least about [[10, 15, 20, 25,]] 30, 35, 40, 45, 50, 75, 100, 150 or 200 consecutive residues of a nucleic acid as set forth in claim 1.

Claim 98 (currently amended): A vector comprising a nucleic acid as set forth in claim 1 or claim 23 [[97]].

Claim 99 (currently amended): The vector of claim 98, [[comprising a]] wherein the vector comprises a viral particle, a baculovirus, a <u>phage</u> [[phase]], a plasmid, a cosmid, a fosmid, a bacterial artificial chromosome, a viral DNA or a P1-based artificial chromosome.

Claim 100 (currently amended): A host cell comprising a nucleic acid as set forth in claim 1 or claim 23 [[97]].

Claim 101 (previously presented): The host cell of claim 100 comprising a eukaryotic cell or a prokaryotic cell.

Claim 102 (previously presented): The host cell of claim 101 comprising a plant cell, a mammalian cell, a fungal cell, a bacterial cell, a yeast cell or an insect cell.

Claim 103 (new): An isolated or recombinant nucleic acid comprising (a) a sequence having at least about 50% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or, (b) a sequence complementary to (a).

Attorney's Docket No.: 09010-010003 / DIVER 1180-2 Applicant: Dan E. Robertson et al.

Serial No.: 09/903,410 : July 10, 2001

: 14 of 26 Page

Claim 104 (new): The nucleic acid probe of claim 67, wherein the oligonucleotide is from about 10 to 50 nucleotides in length.

Claim 105 (new): The nucleic acid probe of claim 84, wherein the oligonucleotide is from about 10 to 50 nucleotides in length.

Claim 106 (new): The isolated or recombinant nucleic acid of claim 1, wherein the esterase activity comprises hydrolysis of ester groups to organic acids and alcohols.

Claim 107 (new): The isolated or recombinant nucleic acid of claim 1, wherein the esterase activity comprises catalysis of a transesterification reaction.

Claim 108 (new): The isolated or recombinant nucleic acid of claim 1, wherein the esterase activity comprises catalysis of an acidolysis reaction.

Claim 109 (new): The isolated or recombinant nucleic acid of claim 1, wherein the esterase activity is thermostable.